Comparison of Profilometric and Microindentation Analyses for Determining the Impact of Saliva on the Abrasion of Initially Eroded Enamel

Steiger-Ronay, Valerie ; Tektas, Sibel ; Attin, Thomas ; Lussi, Adrian ; Becker, Klaus ; Wiedemeier, Daniel B ; Beyeler, Barbara ; Carvalho, Thiago S

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Comparison of profilometric and microindentation analyses for determining the impact of saliva on the abrasion of initially eroded enamel

Valerie Steiger-Ronay*, Sibel Tektas**, Thomas Attin*, Adrian Lussi***, Klaus Becker*, Daniel B. Wiedemeier****, Barbara Beyeler***, Thiago S. Carvalho***

* Clinic of Preventive Dentistry, Periodontology and Cariology, Center of Dental Medicine, University of Zurich, Switzerland
** Private practice, Andelfingen ZH, Switzerland
*** Department of Preventive, Restorative and Pediatric Dentistry, University of Bern, Switzerland
**** Statistical Services, Center of Dental Medicine, University of Zurich, Switzerland

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Correspondence:

Dr. Valerie Steiger-Ronay

Clinic of Preventive Dentistry, Periodontology and Cariology

Plattenstrasse 11

8032 Zürich

Switzerland

Phone +41 44 634 32 84

Fax: +41 44 634 43 08

E-mail valerie.steiger@zzm.uzh.ch

Declaration of interest

The authors declare no conflicts of interest.

Abstract

The aim of this *in vitro* study was to investigate the impact of saliva on the abrasion of eroded enamel using two measuring methods. A total of 80 bovine enamel specimens from 20 bovine incisors were allocated to four experimental groups (n = 20 specimens per group). After baseline surface microhardness (SMH) measurements and profilometry all specimens were subjected to erosion (2 min, 1% citric acid, pH: 3.6, 37° C). SMH was determined again, and the depths of the Knoop indentations were calculated. Thereafter, specimens were incubated in human saliva (group 1 - no incubation / control, group 2 - 0.5 h, group 3 - 1 h, group 4 - 2 h) before toothbrush abrasion was performed. After final SMH measurements and profilometry, indentations were re-measured and surface loss was calculated. SMH did not return to baseline values regardless of the length of saliva incubation. Further, an irreversible substance loss was observed for all specimens. With the indentation method, significantly ($p < 0.05$) more substance loss was found for controls (least square means ± standard error of 198 ± 19 nm) than for groups 2 to 4 (110 ± 10, 114 ± 11, and 105 ± 14 nm). Profilometric assessment showed significantly more substance loss for controls (122 ± 8 nm) than for group 4 (106 ± 5 nm). Intra-class correlation for inter-rater reliability between measurement methods was low (0.21, CI: 0.1-0.3), indicating poor agreement. Exposure of eroded enamel to saliva for up to 2 h could not re-establish the original SMH. Amount of measured substance loss depended on the measurement method applied.

Introduction

Tooth erosion is defined as acid-induced hard tissue loss occurring without the involvement of microorganisms [Eccles, 1979]. Acidic attacks lead to an irreversible loss of superficial enamel layers causing a partial demineralization and softening of the tooth surface. Due to this softening, the eroded tooth surface is more prone to abrasion through mechanical action such as tooth brushing [Davis and Winter, 1980, Attin et al., 1997].

Various techniques have been proposed for the evaluation of dental hard tissue alterations as caused by erosion and abrasion. Surface microhardness measurement constitutes a common approach to determine changes in surface hardness and tooth substance loss. Erosive enamel dissolution is associated with a softening of the surface. The indentation depth of a diamond tip with known geometrical dimensions that is inserted with a certain load depicts this structural change [Attin and Wegehaupt, 2014]. Enamel abrasion can subsequently be determined by differences between the indentation depths before and after abrasion [Jaeggi and Lussi, 1999, Joiner et al., 2004b].

The recording of the enamel topography by surface profilometry constitutes another suitable way of identifying erosive-abrasive substance removal of dental hard tissues [Slop et al., 1983]. In contact profilometry, a map of the tooth surface morphology is generated by a stylus gauging the enamel surface with a known force. Erosive-abrasive substance removal can be determined by comparison of baseline and post-treatment surface profiles.

It has been proposed that the surface demineralization of enamel, as caused by erosive substances, may be remineralized through interaction with saliva [Amaechi and Higham, 2001]. Therefore, patients have been advised not to brush their teeth immediately after consumption of erosive foods or drinks [Davis and Winter, 1980, Edwards et al., 1998] in order to minimize tooth substance loss. This recommendation was confirmed by various *in vitro* and *in situ* studies, which showed that an acidic attack immediately followed by an abrasion leads to a greater enamel loss than if the abrasion takes place after a certain latency period [Jaeggi and Lussi, 1999, Attin et al., 2000, Attin et al., 2001]. Other studies, however, could not demonstrate an advantage of an extended waiting period of two [Ganss et al., 2007] or more hours [Wiegand et al., 2008b, Lussi et al., 2014] between the erosive attack and tooth brushing to increase the abrasion resistance of the enamel. Therefore, there is still some controversy concerning the optimal time interval between the consumption of acidic food or beverages and subsequent tooth brushing. Consequently, the aim of the present study was to evaluate whether a latency period for up to two hours in human saliva results in decreased abrasion of erosively treated enamel specimens. The null hypothesis was that prolonged saliva

contact does not lead to decreased erosive-abrasive enamel loss. This study also focused on the two measurement methods applied, i.e. indentation method as well as surface profilometry, for determining the erosive/abrasive substance loss to investigate their influence on the study result.

Materials and Methods

Experimental procedure

Figure 1 illustrates the experimental procedure. Eighty enamel specimens (made from twenty teeth, i.e. four specimens per tooth) were assigned to four groups in a way, that one specimen from each tooth was included in each group. Baseline profilometry and surface microhardness (SMH) measurements were carried out, followed by erosion of the specimens. SMH was determined again, and baseline Knoop indentation depth measurements were calculated. Thereafter, specimens were incubated in human saliva for different time spans according to their group allocation: no saliva contact (group 1, control), 0.5 h (group 2), 1 h (group 3) and 2 h of incubation (group 4). The four specimens originating from one tooth were treated with the saliva of the same donor before being allocated to the four experimental groups. This resulted in evenly distributed baseline conditions. In order to reduce the number of necessary donors, one donor delivered saliva for the incubation of two bovine teeth. All specimens were abraded before final SMH, indentation and profilometric analyses were performed. Surface loss was determined by indentation method through measurement of the indentation before toothbrush abrasion, re-measurement thereof after toothbrush abrasion and calculation of the difference. Tooth substance loss was further assessed by profilometry through superimposition of baseline and final profiles and calculation of average loss per profile.

Saliva collection

Prior to the study start, ethical approval was obtained from the Cantonal Ethics Commission Zurich (KEK-ZH No. 2015-0064). Ten healthy voluntary saliva donors who provided their written informed consent participated in the study. Any of the following factors led to study exclusion: smoking, intake of medication, hyposalivation (< 2.0 ml stimulated saliva / min), insufficient buffering capacity (< 0.07 ml HCl 0.1 M / ml oral fluid to pH 7.5), pregnancy or breastfeeding. For the experiments, each donor provided 10 ml of stimulated saliva by chewing a paraffin block during 5-10 min. Saliva collection was performed in the morning of the experiment; thereafter saliva was stored cooled (4 °C) in test tubes. One to two hours

elapsed between saliva collection and incubation of the specimens. It was not allowed to eat or drink caffeinated drinks for 2 h before saliva collection.

Salivary flow rate, pH-values (pH-meter 827; Methrom, Herisau, Switzerland), buffering capacity (0.1 M HCl, Impulsomat 614; Methrom, Herisau Switzerland) and calcium content (atomic absorption spectroscopy, contrAA® 300, Analytik Jena, Jena, Germany) of the stimulated saliva were analyzed as described elsewhere [Wiegand et al., 2008a]. Further, phosphate content was assessed [Attin et al., 2005a]. All determined values were within normal range for all donors. Enamel specimens were treated with the saliva of just one donor. This resulted in each of the ten donors providing saliva for the treatment of two teeth, i.e. two specimens per group (n = 20) since the four specimens made from one tooth were distributed to the four experimental groups.

Enamel specimen preparation

Specimens were obtained from 20 intact bovine incisors, which were stored in 0.1% thymol solution. After separation of the crown from the root, four circular enamel specimens with a diameter of 3 mm were prepared out of the labial surface of each crown using a water-cooled diamond core drill. Enamel specimens were embedded in acrylic resin blocks (6 mm in diameter, Paladur, Heraeus Kulzer, Hanau, Germany). All resin blocks had a notch to ensure exact repositioning of the specimens for the profilometric measurements. The enamel surfaces were ground with water-cooled silicon carbide paper discs (# 1200, # 2500, # 4000, Gekko-Papier, Struers, Birmensdorf, Switzerland), followed by fine polishing with a 3 µm and 1 µm diamond abrasive under constant cooling (LaboPol-6, Struers). Specimens were stored in tap water.

Baseline surface wear measurements

Surface microhardness analysis

Surface microhardness (SMH) was determined for all specimens: a Knoop diamond under a load of 50 g and a dwell time of 20 s was used to perform six different indentations with a set distance of 25 µm. Indentations were performed with their long axis parallel to the vertical borders of the ground enamel area. Initial SMH was calculated from the average value of the six indentations, which were measured using the optical analysis system of the hardness measurement device (Microhardness tester, Walter Uhl, Asslar, Germany). Surface microhardness analyses were performed at baseline, after erosion and after abrasion.

Profilometric analysis

For each specimen, five baseline profiles with a set distance of 250 µm between each profile were recorded with a profilometer (Perthometer S2; Mahr, Göttingen, Germany). A custom-made jig ensured the exact repositioning of the specimens for the baseline and final profilometric measurements.

Erosive challenge

Specimens were eroded for 2 min in 50 ml of 1% citric acid (pH: 3.6) in a water bath tempered at 37° C under agitation (75 rotations / min). The specimens were then rinsed with tap water for 30 s and dried with compressed air.

Saliva incubation

Depending on group allocation (group 1 - no saliva, groups 2 - 30 min, group 3 - 1 hour and group 4 - 2 hours of saliva contact), specimens were placed individually into 1 ml of saliva within a water bath at 37 ° C under agitation (95 rotations / min). In group 4 the saliva was replaced by fresh saliva after one hour. Thereafter specimens were rinsed with tap water for 30 s and were ready for abrasion.

Abrasion

For a controlled abrasion, specimens were mounted individually in an automatic brushing machine (Syndicad, Munich, Germany), which contained freshly prepared toothpaste slurry. The slurry consisted of a fluoridated toothpaste (0.14 % fluoride as amine fluoride, Elmex Kariesschutz®, GABA, Basel, Switzerland; RDA 65 ± 3 [Tawakoli et al., 2015]) and tap water at a ratio of 1:2 (w/w). Each specimen was abraded with 100 brush strokes by an American Dental Association (ADA) reference manual toothbrush with a load force of 2 N in reciprocating motion (120 strokes per min). After brushing, specimens were rinsed with deionized water and dried with compressed air for the following final measurements.

Final surface wear measurements

<u>Surface loss calculation using the indentation method</u>

For the indentation method, substance loss was calculated by re-measuring the lengths of six indentations made with the Knoop diamond, as described previously. We measured the lengths of the 6 indentations made soon after the erosive challenge, and re-measured their lengths immediately after the abrasion. The difference in the length ($\Delta l = l_{\text{before abrasion}} - l_{\text{after}}$

$_{abrasion}$) of each indentation was then calculated. The average of Δl was calculated, and the difference between these length values (i.e. difference before and after the abrasion) was used to calculate the difference in the depth of the indentions. This difference in depth values corresponds to the amount of surface loss caused during toothbrush abrasion. This difference in depth (Δd) was determined using the geometrical formula: $\Delta d = 0.5\Delta l/\tan 86.25°$ [Ericson and Bratthall 1989; Jaeggi and Lussi 1999], where Δd is the amount of substance loss, and Δl is the difference in the length of the indentations from before and after abrasion.

Surface loss measurement with the Profilometric analyses

Substance loss was also determined by profilometry. After the final profilometry, an exact superimposition of the baseline profiles with the respective final profiles was performed with custom designed software (4D Client, Custom designed software; University of Zurich, Switzerland) to calculate the average loss of substance per profile [Aykut-Yetkiner et al., 2013].

Statistical analysis

SMH values and individual substance loss measurements as measured by the indentation and profilometric methods, respectively, were averaged per tooth specimen and incubation time. Therefore, due to the potential dependency on saliva, the examination of differences between groups was carried out by means of Generalized Estimated Equations (GEE), followed by the calculation of least square means (LS-means) including confidence intervals (95%) and pairwise comparisons, adjusted according to Tukey. Differences with a p-value ≤ 0.05 were considered statistically significant. For the assessment of the two different measurement methods, their intraclass correlation (two-way, consistency) for the inter-rater reliability was calculated, and a Bland-Altman analysis was performed. The statistical program R [R Core Team 2015] and the R packages "geepack" [Højsgaard et al., 2006; [Yan, 2002], “lsmeans” [Lenth, 2016], “irr” [Gamer et al., 2012] and “BlandAltmanLeh” [Lehnert, 2015] were used for calculations.

Results

Surface microhardness

The calculated least square means ± the respective standard error of means of the baseline SMH, SMH after erosion and final SMH after abrasion are shown in Table 1. SMH baseline

values were very comparable within all groups with an overall average hardness of 341 ± 18. After erosion the SMH LS-means of all specimens decreased significantly, but no significant differences were found between the groups. After abrasion, a significant increase in SMH was found for all specimens without returning to baseline values. Final SMH was significantly lower for the control without salivary contact than the other groups that had been incubated in saliva.

Erosive-abrasive tooth substance loss as determined by SMH and profilometry

The intra-class correlation for the inter-rater reliability between the two measurement methods was low with 0.212 (CI: 0.1-0.3), indicating that their assessments of substance loss do not agree. As graphically illustrated in Figure 2 (Bland-Altman diagram), the measurement outcomes of the two methods differed considerably indicating little consistency of the two methods. Although the mean systematic error between the two methods accounted to only around 19 nm (95% CI: 10 nm; 27 nm), the random error was large (about ± 150 nm) and correlated with average substance loss: A linear fit revealed an intercept a=-123 nm and a slope of b=1.16, with both parameters being significantly different from zero.

The results of the indentation method indicate that there was significantly ($p < 0.05$) greater substance loss following abrasion of the tooth samples in the control group than in the groups that were incubated with saliva (Table 2).

Substance loss as determined by contact profilometry (Table 3) was also highest for the control followed by the other groups with saliva contact. Least substance loss was found after 2 hours of saliva incubation, which was significantly different only from group 1 ($p < 0.05$).

Discussion

The basis of the recommendation to postpone tooth brushing after intake of erosive beverages or foods is the assumption that a precipitation of mineral from saliva takes place, which will reharden the acid-softened tooth surface [Ganss et al., 2007]. In the present investigation a tendency was found that the exposure of softened enamel to saliva for up to two hours resulted in lower tooth brush abrasion. Therefore, the null hypothesis that prolonged saliva contact does not lead to decreased erosive-abrasive enamel loss had to be rejected. Interestingly, the extent of this finding depended on the measuring method applied. Nevertheless, an irreversible erosive-abrasive enamel loss was observed for all specimens, irrespective of the length of saliva contact.

These findings are in line with other experimental studies that used human saliva for remineralisation after erosive-abrasive challenges [Collys et al., 1993, Lippert et al., 2004, Hara et al., 2008]. In an *in situ* study by Ganss et al. [Ganss et al., 2007] even a waiting period of up to eight hours between acid exposure and tooth brushing did not result in significant reduction of erosive substance loss. Correspondingly, an *in vitro* study by Lussi and colleagues [Lussi et al., 2014] could also not confirm a reduction of the erosive-abrasive substance loss through exposure of eroded enamel to saliva for up to four hours.

Recent clinical data also corroborates these findings. An observational, cross-sectional study including more than 3000 young adults from seven European countries found no evidence that postponing tooth brushing after breakfast has any effect on the degree of tooth wear [Bartlett et al., 2013]. Furthermore, no clear patterns between timing of tooth brushing after breakfast and tooth hypersensitivity, which is strongly associated with the presence of erosion, were found in the same population [West et al., 2013]. A recent case-control study could also not detect any association between tooth brushing after meals and erosive tooth wear after adjusting for dietary risk factors [O'Toole et al., 2017].

Both measuring methods applied in this investigation, i.e. profilometry [Bartlett et al., 1997, Ganss et al., 2000, Attin et al., 2001] as well as indentation depth analysis [Jaeggi and Lussi, 1999, Joiner et al., 2004a, Lussi et al., 2004] are considered established methods for the assessment of dental hard tissue loss. While profilometry is a direct method, i.e. the enamel surface is scanned directly with a stylus, the indentation analysis derives enamel loss indirectly by calculating the difference between the indentations depths before and after the erosive-abrasive challenge. For the profilometric analyses as used in this investigation the lower limit of measurements (mean+3×standard deviation) was reported at 0.105 µm [Attin et al., 2009], indicating that readings and differences below 0.105 µm were below quantification limit. Reproducibility of repeated measurements was also very high with low variations in the range of 0 ± 0.031 µm [Attin et al., 2009]. For the indentation analysis, an accuracy value of 0.18 µm and precision values of 0.01 µm and 0.08 µm have been reported for repeated measurements (10 measurements) [Gyurkovics et al., 2017]. The procedures of both profilometry as well as indentation analysis as performed in this study were mutually validated by experienced investigators from both university centers involved in order to minimize any methodological bias.

In this investigation the determined tooth substance loss varied depending on the measurement technique applied. Even though the LS-means of groups 2 to 4 were similar, individually measured values as determined by means of indentation technique and

profilometry did not concur. As can be seen from Figure 2, the two measurement techniques differed greatly with respect to capturing enamel loss. Interestingly, the differences between the methods depended strongly on the mean measured enamel loss, implying a systematic bias (cf. regression line). Moreover, the mean difference in measurements between the two techniques was around 19 nm, which was also significantly different from zero (95% CI: 10 nm – 27 nm). Partly responsible for this observation could be the decreased pressure resistance of the softened enamel. Since after erosion the outermost demineralized enamel layer is very susceptible to mechanical forces, such as the Knoop diamond load or the tip of a profilometer stylus, compression and displacement of tooth material may result in a varying degree of measured substance loss.

This was an *in vitro* study where artificial erosive lesions were created on bovine enamel specimens, and therefore has the important advantage of providing a reproducible experimental set-up. Even though *in vitro* models can never completely reflect the intra-oral situation, in-lab administered experiments ensure little variation for both erosion, i.e. duration and pH level, and abrasion, i.e. duration and number of brushing strokes, force of application, toothbrush design and toothpaste [Wiegand and Attin, 2011]. Citric acid was chosen as erosive component because of its widespread use as additive to beverages and foods. In order to minimize any uncontrollable effects of other ingredients pure acid was preferred over acidic beverages. The contact time with the specimens was set to 2 min, after which a gradual surface softening comprising the outer 350 nm of enamel could be expected. This time was established in pre-experiments, which determined the minimum duration necessary for reproducible measurements. Thus, the erosive conditions were chosen in a way, that substance loss was as low as possible and reproducibly measurable in order to enable the questioned rehardening of the surface during saliva contact. The abrasion of specimens was performed in a way, that on intact enamel no measurable substance loss would have occurred.

It has to be taken into consideration, that abrasion and SMH data are interdependent. If the most superficial softened enamel layer is abraded, the newly revealed surface appears harder, even if no remineralization has occurred in this layer. SMH data after abrasion therefore depends on the abraded layer height, but also on a possible remineralisation, which cannot be separated with current measurement methods. We can only speculate about the hardness of the new surface before abrasion and real changes in hardness thereafter.

The application of bovine teeth as substitute for human teeth for research purposes is common due to difficulties in obtaining intact human teeth in sufficient quantity and quality. Furthermore, the larger bovine tooth surface enables the fabrication of multiple specimens out

of one tooth, thus enabling similar base conditions for different test groups when distributed evenly [Wiegand and Attin, 2011]. It has been demonstrated that prismatic human and bovine enamel presents with morphological similarities during the development of erosive lesions [Meurman and Frank, 1991], and behaves similarly to erosive challenges and remineralizing conditions [Koulourides et al., 1986]. In cyclic erosion/abrasion models, however, it was shown that both erosion and erosion–abrasion was higher in bovine than in human enamel [Attin et al., 2007]. This fact needs to be considered when interpreting the amount of hard tissue loss found in this investigation.

The enamel loss in the different groups was variable, as can be seen in the high values of the standard error of means in Tables 2 and 3. These variations among the specimens of the respective groups might be explained by a variable susceptibility of enamel specimens to an erosive challenge [Attin et al., 2005b]. In order to decrease this variation, however, specimens originating from the same bovine incisor were allocated to all four experimental groups. The natural variation in chemical/mechanical properties was thereby evenly distributed among the groups, as indicated by comparable baseline SMH values. The use of human saliva of different donors could have also played an important role in the results. Saliva composition may vary inter- and intra-individually, as well as intraday, and according to the type and duration of stimulation and time [Shellis et al., 2011]. Further, individual salivary factors, such as mucin content, protein composition and viscosity are also determining its protective effect [Nieuw Amerongen et al., 1987]. In order to control for this effect, however, generalized estimated equations were used to calculate least square means.

The amount of measured substance loss was significantly influenced by the choice of measurement method, i.e. indentation method or surface profilometry. Therefore, study results obtained through different measurement methods cannot be directly compared and need to be interpreted with caution. The null hypothesis that prolonged saliva contact leads to decreased erosive-abrasive enamel loss was rejected. Even though saliva exposure of eroded enamel showed a tendency in lower tooth brush abrasion, irreversible substance loss was not prevented. Accordingly, the clinical recommendation to wait with tooth brushing after intake of erosive beverages or foods should be made with caution.

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Author Contributions:

Conceived and designed the experiments: TA, AL, VSR, TSC, KB

Performed the experiments: ST, KB, BB

Analyzed the data: DBW

Wrote the paper: VSR, TSC

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Legends

Table 1

Surface microhardness (SMH) values as least square means ± standard error of means, as measured in the course of the experiments.

Different capital letters indicate significant differences between groups ($p < 0.05$).

Different lowercase letters indicate significant differences within groups ($p < 0.05$).

LS-mean - least square mean, SE - standard error of means.

Table 2

LS-means, standard errors and confidence intervals of the substance loss [nm], as derived from the indentation method.

LS-mean - least square mean, SE - standard error of means, LCI - lower confidence interval, UCI - upper confidence interval, S - significance: different capitals indicate groups with statistically significant differences ($p < 0.05$), as assessed by LS-means of GEE.

Table 3

LS-means, standard errors and confidence intervals of the substance loss [nm], as derived from measurements from profilometric measurements.

LS-mean - least square mean, SE - standard error of means, LCI - lower confidence interval, UCI - upper confidence interval, S - significance: different capitals indicate groups with statistically significant differences ($p < 0.05$), as assessed by LS-means of GEE.

Figure 1

Flow chart of experimental procedure describing the sequence of baseline surface microhardness (SMH) and profilometric measurements, followed by erosion, SMH measurement after erosion, and baseline indentation measurement. Thereafter saliva treatment, abrasion, and final SMH, indentation and profilometric measurements were performed.

Figure 2

Bland-Altman diagram with differences (indentation depth – profilometric analysis) of the two measurement methods plotted against their mean values. Red lines indicate the estimation of the systematic error of 19 nm (95% CI: 10 nm; 27 nm), blue lines indicate the random error

between the limits of agreement of -150 nm (95% CI: -165 nm; -135 nm) and 187 nm (95% CI: 172 nm; 202 nm). A linear fit of the form differences=a*means+b yields the parameters a = -123 and b = 1.16. Both are significantly different from zero ($p<0.001$).

Table 1

Group	Time in saliva [hours]	SMH baseline [LS-mean ± SE]	SMH after erosion [LS-mean ± SE]	SMH final [LS-mean ± SE]
1	0	336 ± 3 ^{A,a}	298 ± 5 ^{A,b}	312 ± 3 ^{A,b}
2	0.5	345 ± 3 ^{A,a}	304 ± 4 ^{A,b}	330 ± 4 ^{B,a}
3	1	340 ± 3 ^{A,a}	299 ± 3 ^{A,b}	325 ± 4 ^{B,a}
4	2	341 ± 5 ^{A,a}	306 ± 6 ^{A,b}	332 ± 6 ^{B,a}

Table 2

Group	**Time in saliva** [hours]	**LS-mean ± SE** [nm]	**LCI**	**UCI**	**S**
1	0	198 ± 19	160	236	A
2	0.5	110 ± 10	90	130	B
3	1	114 ± 11	92	137	B
4	2	105 ± 14	78	131	B

Table 3

Group	**Time in saliva** [hours]	**LS-mean ± SE** [nm]	**LCI**	**UCI**	**S**
1	0	122 ± 8	106	138	A
2	0.5	113 ± 9	96	130	AB
3	1	110 ± 4	103	118	AB
4	2	106 ± 5	97	115	B

20 Bovine Teeth

From each tooth preparation of 4 enamel specimens with allocation of one/group

- Group 1 (n=20)
- Group 2 (n=20)
- Group 3 (n=20)
- Group 4 (n=20)

↓

- SMH (baseline)
- Profilometry (baseline)

↓

Erosion

2 min in 50 ml of 1% citric acid (pH: 3.6) at 37° C

↓

- SMH (after erosion)
- Indentation method (baseline measurement)

↓

Treatment with saliva from 10 individual donors (2 specimens allocated to each donor)

- Group 1 No Saliva (n=20)
- Group 2 30 min (n=20)
- Group 3 1 h (n=20)
- Group 4 2 h (n=20)

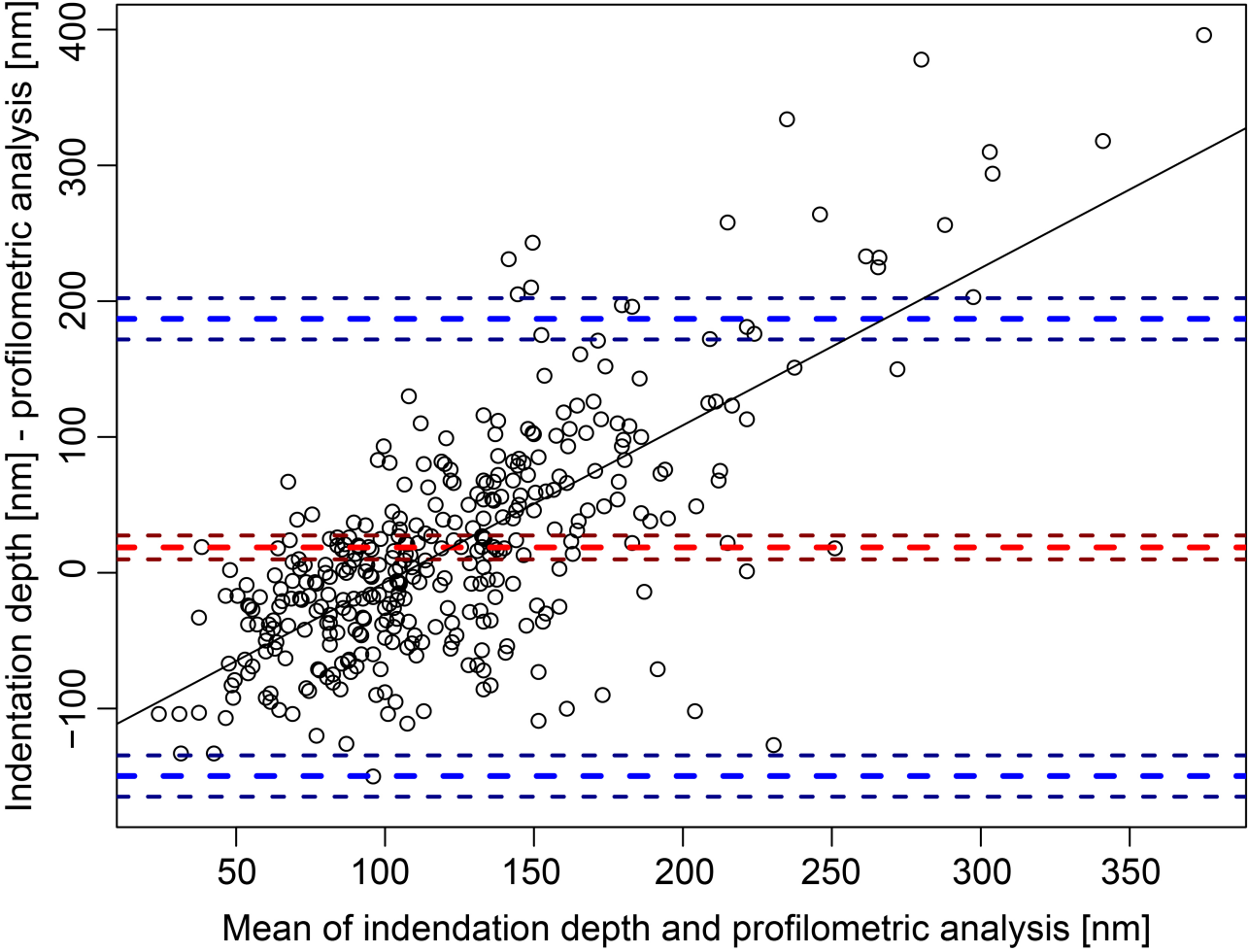
↓

Abrasion

100 toothbrush strokes (load force 2 N) with slurry (Elmex Kariesschutz® toothpaste and water)

↓

- SMH (final)
- Indentation method (final measurement)
- Profilometry (final)


Indentation depth [nm] - profilometric analysis [nm]
400
300
200
100
0
−100
50
100
150
200
250
300
350
Mean of indendation depth and profilometric analysis [nm]